

**EXHIBIT 3**

IN THE UNITED STATES DISTRICT COURT  
FOR THE DISTRICT OF DELAWARE

**TALECRIS BIOTHERAPEUTICS, INC., and**  
**BAYER HEATHCARE LLC**

**[Plaintiff] Plaintiffs,**

**v.**

**BAXTER INTERNATIONAL INC., and**  
**BAXTER HEALTHCARE CORPORATION,**

**Defendants.**

**C.A. No. 05-349-GMS**

**Demand for Jury Trial**

**AMENDED AND SUPPLEMENTAL COMPLAINT**

Talecris Biotherapeutics, Inc. (referred to as “Talecris”) and Bayer Healthcare LLC (referred to as “Bayer”) by [its] their attorneys, for [its] their complaint against Baxter International Inc. and Baxter Healthcare Corporation (collectively referred to as “Baxter”), [alleges] allege as follows:

**NATURE OF THE ACTION**

1. This is an action by Talecris and Bayer against Baxter for:
  - (a) [a declaratory judgment of infringement] Infringement of one or more claims of United States Letters Patent No. 6,686,191 under 35 U.S.C. § 271(g);
  - (b) [a declaratory judgment of infringement] Infringement of one or more claims of United States Letters Patent No. 6,686,191 under 35 U.S.C. § 271(a); and

(c) [a declaratory judgment of infringement] Infringement of one or more claims of United States Letters Patent No. 6,686,191 under 35 U.S.C. § 271(b).

### **THE PARTIES**

2. Plaintiff Talecris Biotherapeutics, Inc. is a corporation organized and existing under the laws of the State of Delaware and maintains a place of business at 4101 Research Commons, 79 TW Alexander Drive, Research Triangle Park, North Carolina 27709.

3. Plaintiff Bayer Healthcare LLC is a limited liability company organized and existing under the laws of the State of Delaware and maintains a place of business at 511 Benedict Avenue, Tarrytown, New York 10591.

[3.] 4. Defendant Baxter International Inc. is a corporation organized and existing under the laws of the State of Delaware, maintains a place of business at One Baxter Parkway, Deerfield, Illinois 60015-4633, and maintains an agent and address for service of process at Corporation Trust Center, 1209 Orange Street, Wilmington, Delaware 19801.

[4.] 5. Defendant Baxter Healthcare Corporation is a corporation organized and existing under the laws of the State of Delaware, maintains a place of business at One Baxter Way, Westlake Village, California 91362, and maintains an agent and address for service of process at Corporation Trust Center, 1209 Orange Street, Wilmington, Delaware 19801.

[5.] 6. Upon information and belief, Baxter Healthcare Corporation is a wholly-owned subsidiary, and is the principal United States operating subsidiary, of Baxter International Inc.

### **JURISDICTION AND VENUE**

[6.] 7. This Court has subject matter jurisdiction of this action, pursuant to 28 U.S.C. §§ 1331 and 1338, in that it involves substantial claims arising under the United States Patent Act, 35 U.S.C. § 1 [.] *et seq.*

[7.] 8. The defendants are subject to personal jurisdiction in this district.

[8.] 9. Venue is proper in this District pursuant to the provisions of [Title 28, United States Code, Sections] 28 U.S.C. §§ 1391 (c), (d) and 1400 (b).

### **THE ‘191 PATENT**

[9.] 10. On February 3, 2004, the United States Patent and Trademark Office issued United States Letters Patent No. 6,686,191 (“the ‘191 patent”), entitled “Preparation of Virally Inactivated Intravenously Injectable Immune Serum Globulin,” upon an application filed by William R. Alonso. The ‘191 patent is assigned to Bayer Healthcare LLC. A copy of the ‘191 patent is attached hereto as [“Exhibit A”] Exhibit A.

[10.] 11. The ‘191 patent [expires] is scheduled to expire on or about July 29, 2020.

[11.] 12. Representative claims of the ‘191 patent include:

Claim 1:

A method of treating a solution of antibodies which may have virus activity, the method comprising

- a) contacting the solution with a trialkylphosphate and a detergent under conditions sufficient to substantially reduce any virus activity and resulting in an increased level of anticomplement activity; and
- b) then incubating the solution of step a) under conditions of controlled time, pH, temperature, and ionic strength, such that the increased anticomplement activity of the solution is reduced to an acceptable level suitable for intravenous administration.

Claim 23:

An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001, a pH between about 3.5 and about 5.0, an antibody concentration of about 10% wt./wt., and a glycine concentration of about 0.2 M.

[12.] 13. Talecris alleges that it is the exclusive licensee of the '191 patent with the right to enforce the patent.

### **FACTUAL ALLEGATIONS**

[13.] 14. On or about April 27, 2005, Baxter obtained approval for a biologics license application ("BLA") from the United States Food and Drug Administration (the "FDA") for the commercial manufacture, use, and sale of a product identified as "Immune Globulin Intravenous (Human), 10% Solution" to be sold under the trade name "GAMMAGARD® LIQUID" (hereinafter "GAMMAGARD LIQUID"). A copy of the letter approving Baxter's BLA is attached to this Complaint as Exhibit B.

[14.] 15. The BLA identifies the manufacturer of GAMMAGARD LIQUID as defendant Baxter HealthCare Corporation. Baxter's BLA approves the manufacture of GAMMAGARD LIQUID at Baxter's facility in Lessines, Belgium.

[15.] 16. Baxter [has] publicly announced its intention to begin sales of its GAMMAGARD LIQUID product within the United States during or before the fourth quarter of 2005 [and has taken immediate and active steps to commence sale in the United States of its GAMMAGARD LIQUID product]. [See Exhibit "C"] See Exhibit C attached and incorporated by reference as if fully set forth herein.

17. On or about September 26, 2005, Baxter publicly announced its launch of sales of its GAMMAGARD LIQUID product within the United States and, upon information

and belief, has commenced sale in the United States of its GAMMAGARD LIQUID product. See Exhibit D attached and incorporated by reference as if fully set forth herein.

[16.] 18. Upon information and belief, Baxter has begun [or shortly intends to begin] importing into the United States and [stockpiling] selling in the United States GAMMAGARD LIQUID product [in anticipation of its intended launch of GAMMAGARD LIQUID sales in the United States].

[17.] 19. The labeling information for GAMMAGARD LIQUID approved by the FDA (the “approved label”) lists three steps of the manufacturing process. The approved label describes, *inter alia*, the steps of manufacturing GAMMAGARD LIQUID as “solvent/detergent (S/D) treatment,” “35 nm nanofiltration,” and “a low pH incubation at elevated temperature.”

[18.] 20. Upon information and belief, the steps and conditions listed by Baxter in the approved label as part of its manufacturing process for GAMMAGARD LIQUID, if practiced in the United States, infringes literally or under the doctrine of equivalents, or will infringe literally or under the doctrine of equivalents, one or more claims of the ‘191 patent.

[19.] 21. Upon information and belief, the importation into the United States, and/or offer for sale, sale, or use within the United States, of Baxter’s GAMMAGARD LIQUID infringes literally or under the doctrine of equivalents, [or] and/or will infringe literally or under the doctrine of equivalents, one or more claims of the ‘191 patent pursuant to 35 U.S.C. §§ 271(a), (b) and (g).

**FIRST CLAIM FOR [RELIEF;  
DECLARATORY JUDGMENT OF] RELIEF: INFRINGEMENT  
OF THE '191 PATENT UNDER 35 U.S.C. § 271 (g)**

[20.] 22. Talecris [realleges] and Bayer reallege paragraphs 1 through [19] 21. above, as if fully set forth herein.

[21.] 23. This count arises under [the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, based upon an actual controversy between the parties] the Patent Laws of the United States, 35 U.S.C. § 1 et seq.

[22.] 24. Upon information and belief, Baxter's GAMMAGARD LIQUID product is made, and is intended to be made, by a process which, if practiced in the United States, infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent.

[23.] 25. Upon information and belief, Baxter International Inc.'s importation into the United States and/or future importation, offer for sale, sale, or use within the United States of Baxter's GAMMAGARD LIQUID product infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent pursuant to 35 U.S.C. § 271(g).

[24.] 26. Alternatively, upon information and belief, Baxter Healthcare Corporation's importation into the United States and/or future importation, offer for sale, sale, or use within the United States of Baxter's GAMMAGARD LIQUID product infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent pursuant to 35 U.S.C. § 271(g).

[25.] 27. Alternatively, upon information and belief, Baxter International Inc. and Baxter Healthcare Corporation's joint importation into the United States and/or future

importation, offer for sale, sale, or use within the United States of Baxter's GAMMAGARD LIQUID product infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent pursuant to 35 U.S.C. § 271(g).

28. Upon information and belief, Baxter's foregoing acts of infringement of the '191 patent were, and continue to be, willful and deliberate.

[26.] 29. Talecris [will be] and Bayer are, and will continue to be, irreparably harmed if Baxter is not enjoined from infringing one or more claims of the '191 patent.

30. As a result of Baxter's infringement, Talecris and Bayer have suffered, and continue to suffer, monetary damages in an amount yet to be determined because of the above-described acts of infringement, and will continue to suffer damages in the future unless Baxter's infringing activities are enjoined by this Court.

31. Talecris and Bayer are entitled to damages adequate to compensate for the infringement, but in no event less than a reasonable royalty.

**SECOND CLAIM FOR [RELIEF;  
DECLARATORY JUDGMENT OF] RELIEF; INFRINGEMENT  
OF THE '191 PATENT UNDER 35 U.S.C. § 271 (a)**

[27.] 32. Talecris [realleges] and Bayer reallege paragraphs 1 through [26] 31, above, as if fully set forth herein.

[28.] 33. This count arises under [the Declaratory Judgment Act, 28 U.S.C. §§ 2201 and 2202, based upon an actual controversy between the parties] the Patent Laws of the United States, 35 U.S.C. § 1 et seq.



[29.] 34. Upon information and belief, Baxter International Inc.'s importation into the United States and/or future importation, offer for sale, sale, or use within the United States of Baxter's GAMMAGARD LIQUID product infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent pursuant to 35 U.S.C. § 271(a).

[30.] 35. Alternatively, upon information and belief, Baxter Healthcare Corporation's importation into the United States and/or future importation, offer for sale, sale, or use within the United States of Baxter's GAMMAGARD LIQUID product infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent pursuant to 35 U.S.C. § 271(a).

[31.] 36. Alternatively, upon information and belief, Baxter International Inc. and Baxter Healthcare Corporation's joint importation into the United States and/or future importation, offer for sale, sale, or use within the United States of Baxter's GAMMAGARD LIQUID product infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent pursuant to 35 U.S.C. § 271(a).

37. Upon information and belief, Baxter's foregoing acts of infringement of the '191 patent were, and continue to be, willful and deliberate.

[32.] 38. Talecris [will be] and Bayer are, and will continue to be, irreparably harmed if Baxter is not enjoined from infringing one or more claims of the '191 patent.

39. As a result of Baxter's infringement, Talecris and Bayer have suffered, and continue to suffer, monetary damages in an amount yet to be determined because of the

above-described acts of infringement, and will continue to suffer damages in the future unless Baxter's infringing activities are enjoined by this Court.

40. Talecris and Bayer are entitled to damages adequate to compensate for the infringement, but in no event less than a reasonable royalty.

**THIRD CLAIM FOR [RELIEF;] RELIEF:  
INDUCING INFRINGEMENT  
OF THE '191 PATENT UNDER 35 U.S.C. § 271 (b)**

[33.] 41. Talecris [realleges] and Bayer reallege paragraphs 1 through [32] 40, above, as if fully set forth herein.

42. This count arises under the Patent Laws of the United States, 35 U.S.C. § 1 *et seq.*

[34.] 43. Baxter International Inc. infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent under 35 U.S.C. § 271(b) by actively inducing Baxter Healthcare Corporation to infringe one or more claims of the '191 patent.

[35.] 44. Alternatively, Baxter Healthcare Corporation infringes literally or under the doctrine of equivalents, and/or will infringe literally or under the doctrine of equivalents, one or more claims of the '191 patent under 35 U.S.C. § 271(b) by actively inducing Baxter International Inc. to infringe one or more claims of the '191 patent.

45. Upon information and belief, Baxter's foregoing acts of infringement of the '191 patent were, and continue to be, willful and deliberate.

[36.] 46. Talecris [will be] and Bayer are, and will continue to be, irreparably harmed if Baxter is not enjoined from infringing one or more claims of the '191 patent.

47. As a result of Baxter's infringement, Talecris and Bayer have suffered, and continue to suffer, monetary damages in an amount yet to be determined because of the above-described acts of infringement, and will continue to suffer damages in the future unless Baxter's infringing activities are enjoined by this Court.

48. Talecris and Bayer are entitled to damages adequate to compensate for the infringement, but in no event less than a reasonable royalty.

**WHEREFORE**, Talecris and Bayer request the following relief:

- A.** A [declaratory] judgment that Baxter's GAMMAGARD LIQUID Immune Globulin Intravenous (Human) is made according to the processes claimed in the '191 patent, and that its importation into the United States and/or its offer for sale, sale, and/or use in the United States is, or will imminently constitute, an infringement, literally or under the doctrine of equivalents, of one or more claims of the '191 patent;
- B.** A judgment permanently enjoining Baxter International Inc. and Baxter Healthcare Corporation from making, using, selling, offering to sell, and importing into the United States GAMMAGARD LIQUID Immune Globulin Intravenous (Human) prior to the expiration date of the '191 patent;
- C.** A judgment permanently enjoining Baxter International Inc. and Baxter Healthcare Corporation from inducing infringement of the '191 patent prior to the expiration date of the '191 patent;
- [D.]** Attorneys' fees in this action under 35 U.S.C. § 285;]

- D. An accounting for damages arising from Baxter's acts of infringement and inducing infringement, including profits made by Baxter and lost by Talecris and Bayer as a result of Baxter's infringing activities;
- E. An award of damages pursuant to 35 U.S.C. § 284, including treble damages for willful infringement as provided by 35 U.S.C. § 284, with interest;
- F. A finding that this is an exceptional case and an order awarding reasonable attorneys fees to Talecris and Bayer pursuant to 35 U.S.C. § 285;
- [E.] G. An award of costs [Costs] and expenses in this action; and
- [F.] H. Such further and other relief as this Court may deem just and proper.

Respectfully submitted,  
**CONNOLLY BOVE LODGE & HUTZ LLP**

DATED: [June 1, 2005] May 5, 2006

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**EXHIBIT A**



US006686191B1

(12) **United States Patent**  
**Alonso**

(10) **Patent No.:** **US 6,686,191 B1**  
(45) **Date of Patent:** **Feb. 3, 2004**

(54) **PREPARATION OF VIRALLY INACTIVATED  
INTRAVENOUSLY INJECTABLE IMMUNE  
SERUM GLOBULIN**

(75) Inventor: **William R. Alonso**, Cary, NC (US)

(73) Assignee: **Bayer HealthCare LLC**, Tarrytown,  
NY (US)

(\*) Notice: Subject to any disclaimer, the term of this  
patent is extended or adjusted under 35  
U.S.C. 154(b) by 1772 days.

(21) Appl. No.: **08/532,211**

(22) Filed: **Sep. 22, 1995**

(51) Int. Cl.<sup>7</sup> ..... **C12N 7/04; A61K 39/395;  
A61K 39/40; A61K 39/42**

(52) U.S. Cl. .... **435/236; 424/176.1; 424/177.1;  
424/130.1**

(58) Field of Search ..... **530/390.1, 390.5,  
530/386, 387.1; 424/176.1, 177.1, 130.1;  
435/236**

(56) **References Cited**

**U.S. PATENT DOCUMENTS**

4,396,608 A \* 8/1983 Tenold  
4,540,573 A \* 9/1985 Neurath et al.  
4,762,714 A \* 8/1988 Mitra et al.

**OTHER PUBLICATIONS**

Joy Yang, Y.H. et al. "Antibody Fc functional activity of  
intravenous immunoglobulin preparations treated with sol-  
vent-detergent for virus inactivation" Vox Sang, vol. 67, pp.  
337-344, May 17, 1994.\*

\* cited by examiner

*Primary Examiner*—Yvonne Eyler

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Hutz LLP

(57) **ABSTRACT**

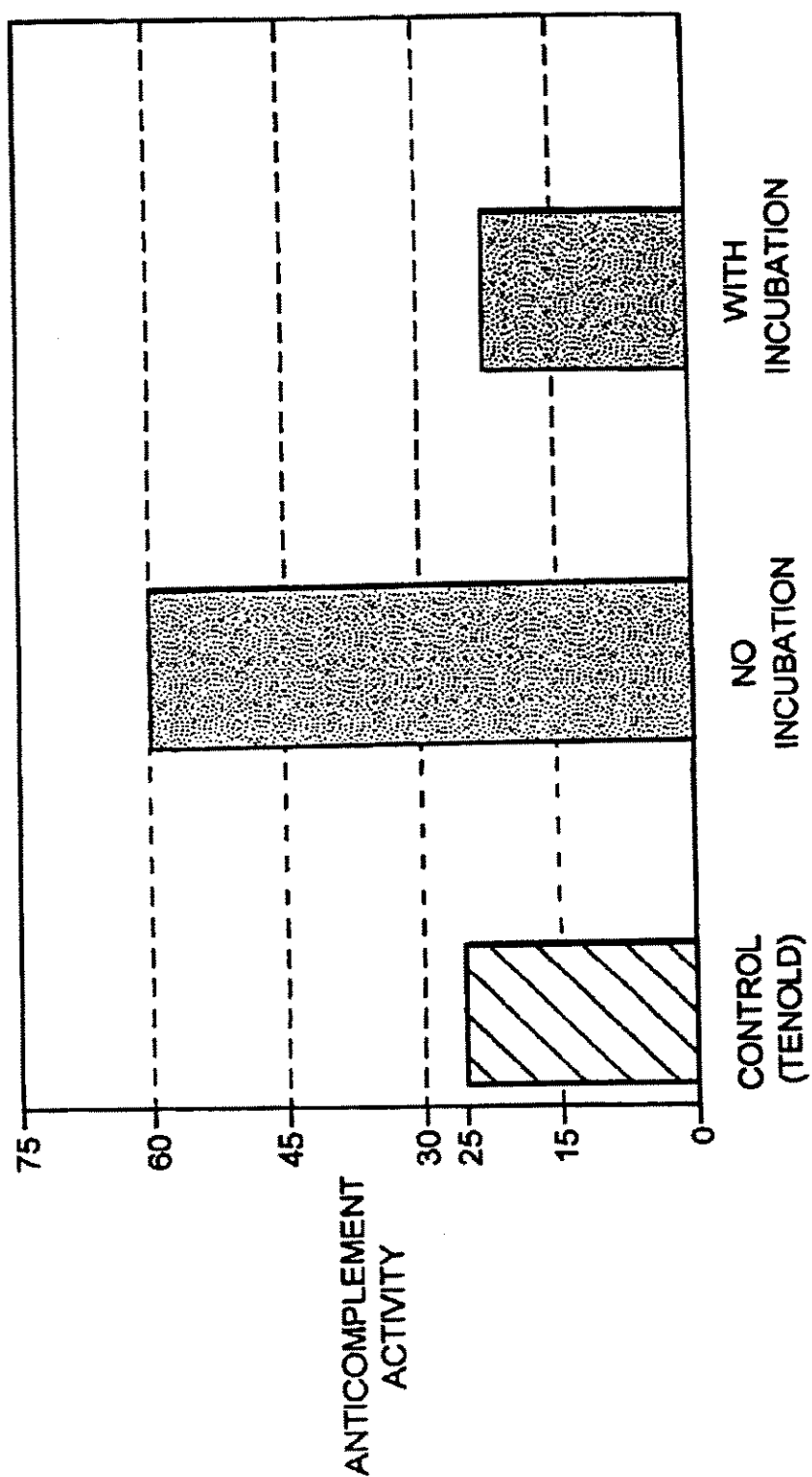
Method of reducing the anticomplement activity (ACA)  
resulting from viral inactivation treatment of a solution of  
antibodies, the method comprising contacting the solution  
with a trialkylphosphate, such as tri-n-butyl phosphate, and  
a detergent, such as sodium cholate, under conditions suf-  
ficient to reduce substantially the virus activity, and then  
incubating the solution under controlled conditions of time,  
pH, temperature, and ionic strength such that the anti-  
complement activity is reduced to an acceptable level. In a  
preferred embodiment, the ACA is reduced to less than 60  
CH<sub>50</sub> units/mL, the incubation is for at least about ten days  
at a pH from 3.5 to 5.0, the temperature is maintained within  
a range of 2 to 50° C., and the ionic strength of the solution  
is less than about 0.001 M.

**24 Claims, 1 Drawing Sheet**

U.S. Patent

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## PREPARATION OF VIRALLY INACTIVATED INTRAVENOUSLY INJECTABLE IMMUNE SERUM GLOBULIN

### BACKGROUND OF THE INVENTION

#### 1. Field

This invention generally deals with an intravenously injectable immunoglobulin product, and more specifically deals with an intravenously injectable immune serum globulin (IGIV) which has been subjected to a virus inactivation step and which has a low level of anticomplement activity.

#### 2. Background

Early pharmaceutical preparations of immune serum globulins could not be administered intravenously due to an unacceptably high incidence of adverse reactions. These adverse reactions were associated with a decrease in serum complement levels, apparently caused by complement binding to the administered gamma globulin. (1) The ability of gamma globulin to bind complement, or its anticomplement activity (ACA), is greatly increased as a result of denaturation brought about during the fractionation procedure. Several approaches have been taken to address the problem of rendering ISG safe for intravenous administration. (See (2) and references therein). Tenold reported a method of preparing an immune serum globulin (ISG) with low ACA which could be administered by intravenous injection. (2, incorporated herein by reference). The Tenold '608 process requires formulating the ISG at low ionic strength (preferably less than about 0.001) and at low pH (3.5-5.0).

Other methods of preparing intravenously injectable immune serum globulin (IGIV) have been reported, including stabilizing with carbohydrates such as maltose (3). A process including incubation of ISG at pH 4.0 at 37° C. (4) results in a product with low ACA which may be administered by intravenous injection; however, upon storage the product regains its high ACA. IGIV has also been prepared by covalent modification of the ISG, for example by proteolysis (5) or by reduction of disulfide linkages followed by reaction with a blocking agent (1,6).

Antibody preparations, since they are isolated blood products, have an inherent hazard of transmitting virally-mediated diseases. Inactivation of viruses is an important step in producing safe and effective blood products. U.S. Pat. No. 4,540,573 to Neurath et al., which is incorporated herein by reference, describes a viral inactivation process using a trialkyl phosphate and detergent process (hereinafter, the solvent/detergent process, or SD process). (7) That solvent/detergent method has gained acceptance as being efficacious in the inactivation of lipid-enveloped viruses with limited adverse effects on biological activity or blood product profile. (8, 15; See also 12 for a discussion of various viral inactivation processes).

Current antibody preparations on the market generally have been regarded as safe with respect to viral contamination. (9) This is thought to be due to features of the fractionation processes used to isolate these blood products. However, it would be desirable to further ensure the safety of the antibody preparations by including a distinct viral inactivation step in the production process. Successful reduction of viral activity in an IGIV solution was reported using several different methods of viral inactivation for a variety of viruses. (16, 17) A process for preparation of immunoglobulins substantially free of retrovirus has been reported involving incubation of ISG under controlled conditions of time, temperature, and pH. The process entails

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isolating ISG via a cold ethanol plasma fractionation process and then storage of the ISG at one of two storage conditions: (a) at pH  $\leq 4.25$  at a temperature of 27° C. for at least three days, or (b) at pH  $\leq 6.8$  at a temperature of 45° C. for at least six hours. (10).

We have found that using the SD process to treat ISG preparations, especially those subsequently formulated according to the Tenold '608 patent, results in a product with an acceptable viral inactivation but with unacceptably high levels of ACA. Elevated ACA levels were always detected at the sterile bulk stage (i.e., after compounding as 5% or 10% IGIV and filtration with 0.2  $\mu$ m sterile filters) of all tri-n-butyl phosphate (TNBP)/detergent treated IGIV preparations regardless of process scale. Preparations of ISG with high ACA levels are not suitable for intravenous injection and instead must be administered via other routes, e.g. intramuscular (IM) injection. However, IGIV preparations are more desirable since they are immediately available in the bloodstream and are not subject to loss associated with IM injection. It is thus desirable to have an IGIV product which is both low in ACA and has been subjected to a viral inactivation step.

### SUMMARY OF THE INVENTION

The invention is a method for producing an intravenously injectable immune serum globulin (IGIV) preparation with low anticomplement activity which has been chemically treated to render it substantially free of lipid-enveloped viruses. The method comprises a solvent/detergent viral inactivation step followed by an incubation step. We have discovered that the incubation step is necessary to achieve an acceptable level of ACA low enough to allow the ISG to be administered by intravenous injection. The incubation step should be conducted under controlled time, pH, temperature, and ionic strength. Preferably, the pH should be maintained between about 3.5 and about 5.0, the temperature should be within a range of about 2 to about 50° C., and the ionic strength should be less than about 0.001M. In a preferred embodiment the ACA of the ISG preparation decreases gradually over a period of at least about ten days when the ISG is maintained at a pH of about 4.25 at low ionic strength (less than about 0.001M) and the viral inactivation step (in a model system) results in a substantial reduction (i.e. at least 4 logs) in the titer of lipid enveloped viruses.

### BRIEF DESCRIPTION OF THE FIGURE

FIG. 1 shows a comparison of the typical average observed ACA levels of 5% IGIV solutions treated according to the SD process and with or without the follow-up incubation of the present invention.

### SPECIFIC EMBODIMENTS

#### Materials and Methods

The starting material for the process of this invention is unmodified human immune serum globulin. In the specification and claims the term "immune serum globulin" is used to define the substance also referred to in the literature variously as gamma globulin, IgG and immunoglobulin G. It consists predominantly and preferably of at least about 85 percent of the 7S species of gamma globulin, which has a molecular weight of about 160,000. Any remainder is preferably 9S species, with a molecular weight of about 300,000. Both standard immune and hyperimmune serum globulins, e.g., tetanus, rabies and hepatitis immune serum globulins,



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can be employed, the solvent/detergent treated product being immune and hyperimmune ISG, respectively. Thus, a suitable starting material for the process of this invention is Cohn's Fraction II or Fraction III filtrate. (See Refs. 13, 14.)

Fraction II, by ultracentrifugation studies, is predominantly (about 85 percent) the 7S (sedimentation constant of 7) species of gamma globulin with an average molecular weight of 160,000. The remaining protein is essentially 9S material with a M.W. of about 300,000. Wet Fraction II paste (approximately 30 percent solids) is commonly lyophilized to obtain dry ISG powder which is then dissolved and prepared for intramuscular injection as a 16.5 percent sterile solution. Either the wet Fraction II paste or the dry ISG powder is a suitable starting material for the process of this invention.

Gamma globulin obtained by any process which has essentially the same composition of protein components as found in the Cohn Fraction II or Fraction III filtrate can be used as starting material in the present process. Both standard immune serum globulin and hyperimmune serum globulin can be employed as starting materials. As is well known, the latter is produced from plasma or serum obtained from selected donors who have much higher titers for a specific antibody than is normally found in the average population. These donors have either been recently immunized with a particular vaccine or else they have recently recovered from an infection or disease. These high titer sera or plasmas are pooled and subjected to the usual Cohn fractionation procedures up to the point of isolating Fraction II.

Furthermore, because the amount of antibody required to achieve a desired immunological response is substantially less when administered intravenously, it will be apparent the intravenous dose will be substantially less than the intramuscular dose which will produce the same serum antibody titer. Thus, the dose of intramuscular ISG and hyperimmune serum globulin must be higher than that required to achieve the same serum antibody titer when globulin of the same antibody activity is administered intravenously.

The starting wet paste or lyophilized powder is dissolved in a volume of water or other physiologically-acceptable carrier to provide a protein solution of a concentration of about 0.5–20% preferably about 5 to 10 percent. If Fraction III filtrate is employed, the aqueous solution must be concentrated by conventional techniques to the desired protein concentration. Any protein concentration may be used in this method; however, the above range is preferred from a practical standpoint.

After the protein has been dissolved or concentrated, the solution is adjusted to a pH of about 3.5 to 5.0 preferably about 3.8 to 4.2, by addition of a physiologically-acceptable acid such as hydrochloric acid. In general, the pH is adjusted to a point whereat the monomeric material in the protein solution is maintained at a maximum. However, the pH must not be so low as to result in gelation. The temperature should not be harmful to the ISG material. Good results are obtained within the temperature range of about 0–20° C. It is not necessary to hold the so-adjusted material for any period of time prior to the next step; however, the material may be held, if desired, without detrimental effects.

The protein solution at the appropriate pH (preferably 3.8–4.2) may be diafiltered with at least 4 volume exchanges of water to reduce the alcohol concentration from approximately 17% (Filtrate III) to about 2% alcohol. The efficacy of solvent/detergent as a viral inactivation method is much better at or above ambient temperatures; however, high

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concentrations of alcohol at these temperatures will denature the IgG molecules. Thus, this inactivation must be performed in low alcohol concentration.

Next, the protein concentration of the so-treated material is adjusted to the level desired for incubation with TNBP/detergent, generally less than 10% protein for maximum viral inactivation. This adjustment is accomplished by conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Prior to addition of TNBP/detergent, the pH may be adjusted within a wide range, depending on the detergent to be used. With Tween 80, the pH may be as low as 3.5, where the IgG starts becoming unstable. With cholate, the pH is adjusted to within the range of 5.0–6.4, preferably about 5.6, prior to addition of TNBP/detergent. Satisfactory cholate solubility during incubation was achieved by adjusting the immunoglobulin solutions to a pH of 5.5 or higher prior to addition of TNBP and sodium cholate. Adjusting the IgG solution to pH values lower than 5.5 is not suitable because the solubility of sodium cholate is highly dependent on pH (cholic acid pK=6.4), with poor solubility at pH 5.5 or lower. Furthermore, maximum viral inactivation during incubation with TNBP/cholate was observed at pH values less than 6.0 in experiments which employed model viruses spiked into IgG solutions. The inactivation of HIV-1 and BVDV (bovine viral diarrhea virus, which is employed as a model for hepatitis C) was accelerated at pH 5.8, with inactivation to the detection limit occurring in 1–2 hours, whereas inactivation to the detection limit required a minimum of 6 hours when pH 7 conditions were used.

Next, the TNBP/detergent is added to the protein solution (preferably less than 8% [w/w], pH 5.8) mixed thoroughly, and then incubated above ambient temperatures, for example 30° C., with continuous agitation or mixing. Target TNBP/cholate levels for optimal viral inactivation during the incubation step should be >3 mg/mL TNBP and >2 mg/mL cholate as defined by Edwards et al. (8) Moreover, for effective viral inactivation, it is important that the solution is essentially free of particulates in order to facilitate thorough mixing of solvent/detergent and IgG solution. After incubation with TNBP/cholate under these conditions, greater than 5.2 log<sub>10</sub> reduction of HIV-1 and greater than 4.0 log<sub>10</sub> reduction of BVDV were detected.

After completing the incubation which provides the viral inactivation, the solvent and detergent molecules must be removed in order to achieve a final product with low levels of residual TNBP and cholate which would be suitable for intravenous administration. Generally, procedures to remove detergent are also effective in removing TNBP, and vice versa. Very low levels of TNBP and cholate in the final container can be achieved by a combination of filtration, diafiltration and hydrophobic chromatography. After completing the incubation, the majority of cholate (and TNBP) can be removed from the protein solution by filtration, providing the solution had been previously adjusted to a lower pH value such as 4.0, because sodium cholate is sparingly soluble in aqueous solutions at such pH values. Moreover, all processing steps which follow the solvent/detergent incubation are performed at lower pH values (i.e., 4.0) because IgG molecules are more stable at pH values between 3.5–5.0, in low ionic strength solutions. (2) Thus, after incubation with TNBP/cholate, the protein solution is adjusted to approximately pH 4.0 and incubated at 0–8° C. in order to promote cholate precipitation. Next, filtration is employed to remove the precipitated cholate from the IgG solution.

The so-treated solution is diafiltered with at least four volume exchanges of water to reduce the ionic strength and

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to remove additional TNBP and cholate. After or during the above treatment, the pH is measured and maintained within the range of about 3.5–5.0. The protein concentration of the so-treated material is adjusted to 10–30%, usually 13% (w/v) by employing conventional techniques not detrimental to ISG, e.g., ultrafiltration, reverse osmosis, sublimation, evaporation, etc. Again the pH of the preparation is maintained within the range of about 3.5–5.0, preferably about 3.8–4.2.

In the present invention, hydrophobic chromatography is employed to remove the TNBP and cholate not eliminated by the filtration and diafiltration steps, and thus provide a final product with low levels of residual TNBP and cholate which is suitable for intravenous administration. Hydrophobic chromatography is a method for TNBP removal from protein solutions that has fewer drawbacks and limitations than other available methods such as oil extraction, ion exchange or affinity chromatography. In part, this is because the protein of interest (IgG) remains in solution throughout the TNBP removal process. Polystyrene-based resins (typically PLRP-S from Polymer Laboratories, Amherst, Mass.) were used to remove the solvent/detergent from solution, as we have found the polystyrene-based resins to be superior to other resins, such as silica-based C-18 resins.

Next, the ISG preparation is adjusted to 5% or 10% protein, and treated to render it tonic, i.e., to render it compatible with physiological conditions, or render it physiologically acceptable upon injection. In a preferred embodiment, the tonicity is adjusted to about 230 to about 490 mosmol/kg solvent. More preferably, the tonicity range is from about 250 to about 350 mosmol/kg solvent, and most preferably the tonicity range is from about 260 to about 325 mosmol/kg solvent. The 5% formulation (5% IGIV) is made tonic by the addition of 10% maltose. The 10% formulation contains 0.2 M glycine in order to achieve an isotonic preparation without large quantities of sugar. The product with either formulation (Gamimune®N 5% or Gamimune®N 10%) experiences shifts in molecular distribution (antibody aggregation) when the ionic strength of the low pH solution is increased. Therefore, sodium chloride, which is often used to achieve tonicity, should not be used.

The so-treated solution is incubated at pH 4.25 under low ionic strength conditions (NLT 21 days at 20–27° C. preferred) in order to provide a lowering of ACA levels. The ionic strength is determined according to Perrin (18), and in a preferred embodiment the ionic strength should be less than about 0.001M. Elevated ACA levels were always detected at this stage of all TNBP/cholate treated IGIV preparations (regardless of process scale); however, ACA levels are gradually lowered by incubation at pH 4.25 under low ionic strength conditions (Tables 3, 5–7). While there is no strict rule for determining when the ACA level is low enough to be an acceptable level suitable for intravenous administration, IGIV preparations should have ACA levels as low as possible.

The Figure depicts the typical average reduction of ACA observed in 5% IGIV solutions following SD treatment. For a 5% ISG formulation the acceptable level suitable for intravenous administration preferably would be less than about 45 CH<sub>50</sub> units/mL, and more preferably less than about 30 CH<sub>50</sub> units/mL. For a 10% ISG formulation, the acceptable level suitable for intravenous administration preferably would be less than about 60 CH<sub>50</sub> units/mL, and more preferably less than about 45 CH<sub>50</sub> units/mL. As used herein, one unit of ACA activity (one CH<sub>50</sub> unit) is defined as the amount of protein capable of activating 50% of the complement in an optimally titrated complement and red

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blood cell/hemolysis system. The assay measures the amount of complement that is bound by the mixture of standardized amounts of complement and protein. See refs. 19–20 for a discussion of the assay. Briefly, red blood cells that have been sensitized by preincubation with red blood cell antibodies are added to the complement/protein mixture. In the presence of free complement (not already bound by the protein) these sensitized cells will lyse, releasing hemoglobin which can be quantitated as a measure of the degree of lysis. In parallel, sensitized red blood cells are also added to a buffer control-complement mixture, whose degree of lysis is defined as 100%. The difference between the actual amount of complement needed to give 100% lysis and the amount of complement remaining unbound in the presence of protein equals the amount of complement actually bound by the protein, or anticomplement activity.

#### Results

##### Anticomplement Activity of ISG Resulting From Viral Inactivation Process

To establish the effect of the SD viral inactivation process on solutions containing ISG which are formulated according to the Tenold '608 patent, the experiments depicted in Table 1 were performed. The starting material (SM) was Cohn process filtrate III which had been ultrafiltered to about 5% protein and then diafiltered with four volumes of water.

In the control experiment, incubation (–)/SD (–), the SM was not subjected to any incubation or solvent/detergent treatment. In the incubation (+)/SD (–) experiment, the pH of the SM was adjusted to 7.0, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. In the incubation (+)/SD, TNBP & Tween 80 (+) experiment, the pH of the SM was adjusted to 7.0, 3 mg/mL TNBP and 2 mg/mL Tween 80 were added to the solution, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. In the incubation (+)/SD, TNBP & cholate (+) experiment, the pH of the SM was adjusted to 7.0, 3 mg/mL TNBP and 2 mg/mL cholate were added to the solution, the solution was incubated at 30° C. for ten hours, and then the pH was reduced to 4.0. The solutions in each experiment were then diafiltered with four volumes CWFI (cold water for injection) and concentrated by ultrafiltration. After addition of dry maltose to 10% w/v, the 5% IGIV solution (pH 4.25) was filtered through a 0.2 µm filter.

TABLE 1

Anticomplement activity in 5% IGIV produced by variations of the Solvent/Detergent IGIV Process	
	ACA (CH <sub>50</sub> /mL)
Control (no solvent/detergent treatment, no 30° C. incubation)	25
Incubate at 30° C. for 10 hr (no solvent/detergent)	22
Incubate at 30° C. for 10 hr NLT 3 mg/mL TNBP	68
NLT 2 mg/mL Tween 80	
Incubate at 30° C. for 10 hr NLT 3 mg/mL TNBP	>100
NLT 2 mg/mL cholate	

\*These samples were assayed for ACA after final compounding according to the Tenold '608 patent, but they were not incubated at pH 4.25 and 22° C. prior to analysis.

The results listed in Table 1 show that levels of ACA increased in IgG samples after incubation with TNBP/cholate or TNBP/Tween 80. ACA levels were not elevated in IgG samples that were incubated for 10 hr at 30° C. in the

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absence of solvent/detergent. These results suggest that ACA levels of IGIV samples were not elevated by either processing manipulations or incubation for 10 hr at 30° C. in the absence of solvent/detergent.

TABLE 2

Anticomplement activity in 5% IGIV spiked with TNBP/Na cholate	
	ACA (CH <sub>50</sub> /mL)
5% IGIV, no TNBP/cholate	12
5% IGIV with 100 µg/mL TNBP, 100 µg/mL Na cholate	13

Furthermore, spiking experiments (with TNBP and Na cholate, Table 2) have demonstrated that the elevated anticomplement activity levels were not artifacts caused by carrying out the anticomplement assay in the presence of trace levels of TNBP/Na cholate. Thus, using the prior art SD process for viral inactivation of a solution containing ISG, subsequently formulated according to the Tenold '608 patent, yields a product which has high ACA and is unsuitable for intravenous administration. In a similar experiment, SD treated samples which were not incubated (Table 3, Initial Testing) had ACA levels greater than 100 units.

TABLE 3

Reduction in Anticomplement activity of samples previously treated with TNBP/cholate		
ACA (CH <sub>50</sub> /mL)		
Sample	Initial Testing (no incubation)	After incubation 6 wk. @ 5° C. 3 wk. @ 22° C.
RB21872-16	>100	33
RB21872-17	>100	34
RB21872-18	>100	36
RB21872-20	>100	27

However, when duplicate SD treated samples were incubated for extended periods of time (6 weeks at 5° C. and 3 weeks at 22° C.), the level of ACA was markedly reduced (Table 3, after incubation). This led to further investigation of this surprising observation.

#### Aggregate Content of ISG Exposed to TNBP/cholate

The samples of the previous experiment (Table 3, Initial Testing) were analyzed by size exclusion (gel permeation) HPLC immediately after compounding to determine the extent of aggregation of the IGIV at the initial time point. HPLC analysis shows nearly complete monomer content in the samples. (Table 4).

TABLE 4

HPLC analysis of non-incubated 5% IGIV samples (Table 3 Initial)				
Sample	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
RB21872-16, initial	0.140	0.00	99.86	0.00
RB21872-17, initial	0.146	0.00	99.85	0.00
RB21872-18, initial	0.124	0.00	99.88	0.00
RB21872-20, initial	0.172	0.00	99.83	0.00

Previously, high IgG aggregate levels were shown to correlate with high anticomplement activity. However, results from analysis of the samples show the level of ACA

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in the samples to be greater than 100 units. (Table 3, 'Initial Testing') The HPLC analysis shows that the high ACA following the TNBP/cholate treatment was not due to the presence of aggregated IgG molecules.

#### 5 Varied Conditions of Time and Temperature

The SM was the same as in the previous experiment, and experimental conditions were similar with the following changes. The solutions were treated with TNBP/cholate at pH 7.0 and then were compounded to 5% IGIV, 10% maltose, pH 4.25, as above. The ACA was assayed immediately after final compounding, after a first incubation for nine days at 5° C., and after a second incubation for 21 days at either 22° C. or 5° C. The results are presented in Table 5.

TABLE 5

ACA of TNBP/cholate treated IGIV samples	
Sample Point	ACA (CH <sub>50</sub> /mL)
<u>Intermediate Samples</u>	
Initial sterile bulk	>100
Incubated 9 d. @ 5° C.	>100
<u>Final Incubation</u>	
21 d. @ 22° C.	49
21 d. @ 5° C.	71

In the initial sterile bulk sample, which was treated with TNBP/cholate at pH 7.0, the level of ACA was again greater than 100 units for the initial time point, confirming the observations noted in Table 3. Upon incubation at 5° C. for nine days, the ACA remained greater than 100 units. The final incubation step at either 5° C. or 22° C. shows that the reduction in ACA is dependent on temperature, with faster reduction in ACA observed at higher temperatures.

#### Effect of pH During Solvent/detergent Treatment on ACA

ACA levels were evaluated after incubation with TNBP/cholate at pH 5.8 because better viricidal activity was observed at pH values less than 6.0. Generally, the non-incubated sterile bulk samples of material incubated at pH 5.8 had lower ACA levels than the pH 7.0 samples, but the trend of lowering ACA upon incubation was repeated in the pH 5.8 samples. In fact, the ACA levels continue to decrease beyond the 21 day incubation in samples that initially had elevated ACA levels after incubation with TNBP/cholate at pH 5.8 (Table 6). As was previously noted for the samples incubated at pH 7.0, the lowering of ACA was not due to decreasing levels of aggregated IgG molecules because the material treated at pH 5.8 was essentially monomeric IgG prior to 22° C. incubation (HPLC analysis, sample A4, Table 8).

TABLE 6

Sample A4 - ACA upon extended incubation	
Incubation at 22° C. (days)	CH <sub>50</sub> /mL
0	122
10	73
19	55
25	56
28	45
30	40
34	39
41	33



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TABLE 6-continued

Sample A4 - ACA upon extended incubation	
Incubation at 22° C. (days)	CH <sub>50</sub> /mL
48	30
55	29

Similar results were achieved with samples formulated to 10% IGIV, 0.2 M glycine in the sterile bulk stage. Upon incubation at low ionic strength at pH 4.25 for 10 and 21 days, the levels of ACA were seen to decline in both 5% IGIV samples and 10% IGIV samples. (Table 7) The decrease in ACA can thus be observed over a range of ISG concentrations and over a range of pH values for the solvent/detergent treatment. (Tables 3, 5, 7) HPLC analysis (Table 8) of the sterile bulk samples presented in Table 7 confirmed that the elevated ACA levels were not due to aggregation of ISG molecules.

TABLE 7

ACA of samples treated with TNBP/cholesterol at pH 5.8			
Sample	Sterile bulk (day zero) (CH <sub>50</sub> /mL)	10 days incubation at 20–27° C. (CH <sub>50</sub> /mL)	21 days incubation at 20–27° C. (CH <sub>50</sub> /mL)
A1 (5% IGIV)	43	ND	10
A2 (5% IGIV)	31	14	15
A3 (5% IGIV)	44	15	12
A4 (5% IGIV)	122	73	55
B1 (10% IGIV)	>100	48	46
B2 (10% IGIV)	49	36	30
B3 (10% IGIV)	53	ND	37

Taken together, the above results suggest that ISG products which have been subjected to a solvent/detergent viral inactivation process resulting in an undesirable ACA increase can be made suitable for IV administration by incorporating an additional incubation step under the conditions described here to reduce the ACA to an acceptable level.

TABLE 8

HPLC Analysis of sterile bulk samples treated with TNBP/cholesterol at pH 5.8				
Sample	Aggregate (%)	Dimer (%)	Monomer (%)	Fragment (%)
A2	0.140	0.00	99.86	0.00
A3	0.146	0.00	99.85	0.00
A4	0.124	0.00	99.88	0.00

## CONCLUSION

The ACA increase resulting from the solvent/detergent treatment of the IGIV (antibody) solution appears to be an unavoidable secondary effect of TNBP/detergent treatment to inactivate viruses in the solution. I have discovered that by incubating the solution of IGIV at low pH (4.25) and low ionic strength (0.001M) for a relatively long period of time (at least about 10 days), the ACA gradually decreases over the period of incubation.

The prior art discloses a method of producing IGIV (the Tenold '608 patent) using low pH and low ionic strength.

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The Tenold '608 method omits the viral inactivation step, and thus avoids the problem of increased ACA, but the possibility of viral activity remains. Unlike Tenold, incubation is an essential aspect of the present invention for reducing the ACA.

The Neurath et al. '573 patent teaches the solvent/detergent viral inactivation step. However, Neurath '573 does not mention controlling the pH and also does not mention any consequences of the process relating to ACA. Elevated ACA levels were detected at the sterile bulk stage of TNBP/cholesterol treated IGIV preparations. However, ACA levels decreased upon incubation for at least about 10 days at pH 4.25, low ionic strength, and not less than about 20° C. (See Tables 5–7) The prior art describes several approaches to lowering ACA levels of purified IgG preparations, including removal of IgG aggregates. (11) IgG aggregates have been shown to activate the complement system in vivo. (1) In the present invention, however, lowering of IgG ACA was not due to decreasing levels of IgG aggregates because these TNBP/cholesterol treated IGIV preparations contained low levels of aggregated IgG (as measured by HPLC, Tables 4, 8) prior to incubation under such conditions.

It would be desirable to produce substantially virus-free IGIV, but following the prior art results in a product with an unacceptable level of ACA. Note that Tenold '608 states that the product is substantially free of ACA, but use of the SD process in conjunction with Tenold '608 does result in high levels of ACA: experimental results reported here show that treating ISG solutions with the SD process and then formulation according to the Tenold '608 patent leads to a product with high ACA. (See Tables 1, 3, 5–7) The surprising finding reported here is that a follow-up (terminal) incubation step lowers the ACA of the solvent/detergent treated solution. The typical average observed ACA levels of 5% IGIV solutions treated according to the SD process and with or without the follow-up incubation are compared in the Figure. The present invention thus includes a previously unobserved method of reducing the ACA by incubating under controlled conditions of pH, temperature, and ionic strength for a period of time, thus allowing the product to be administered by intravenous injection.

Mitra '714 does not suggest the use of a S/D process but, instead, reports that a relatively brief incubation of an ISG product under similar conditions results in a substantially virus free preparation. (10) However, employing incubation under such conditions to provide a lowering of anticomplement activity is a novel application of these incubation conditions which were previously employed in the IGIV process for inactivation of enveloped viruses.

The newly developed IGIV process reported here, which includes an additional internationally accepted viral inactivation procedure (treatment with TNBP/cholesterol), generates IgG preparations which have low ACA levels and are suitable for IV administration. The major advantage is that an IGIV product with improved safety can be obtained by a two-step process that includes a TNBP/cholesterol treatment for viral inactivation and incubation under conditions that afford low ACA levels that are suitable for IV administration.

The above disclosure is intended to illustrate the invention, and it is thought variations will occur to those skilled in the art. Accordingly, it is intended that the scope of the invention should be limited only by the claims below.

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- 20 Mayer, M. M., *Quantitative C'Fixation Analysis, Complement and Complement Fixation*, in *Experimental Immunochemistry* (Ed. E. A. Kabat and M. M. Meyer, Thomas, Springfield, Ill., 1961), pp. 214-216, 227-228. What is claimed is:
  1. A method of treating a solution of antibodies which may have virus activity, the method comprising
    - a) contacting the solution with a trialkylphosphate and a detergent under conditions sufficient to substantially reduce any virus activity and resulting in an increased level of anticomplement activity; and
    - b) then incubating the solution of step a) under conditions of controlled time, pH, temperature, and ionic strength, such that the increased anticomplement activity of the solution is reduced to an acceptable level suitable for intravenous administration.
  2. The method of claim 1, wherein the anticomplement activity is reduced to less than about 60 CH<sub>50</sub> units/mL.
  3. The method of claim 1, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 45 CH<sub>50</sub> units/mL.
  4. The method of claim 3, wherein the solution comprises about 5% wt./wt. antibody and the anticomplement activity is less than about 30 CH<sub>50</sub> units/mL.
  5. The method of claim 1, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 60 CH<sub>50</sub> units/mL.

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6. The method of claim 5, wherein the solution comprises about 10% wt./wt. antibody and the anticomplement activity is less than about 45 CH<sub>50</sub> units/mL.
7. The method of claim 1, wherein the incubation is for at least about ten days.
8. The method of claim 1, wherein the pH is maintained within a range of about 3.5 to about 5.0.
9. The method of claim 1, wherein the temperature is maintained within a range of 2° C. to 50° C.
10. The method of claim 1, wherein the ionic strength is less than about 0.001 M.
11. The method of claim 1, wherein at least about 99% of the antibodies are monomeric.
12. The method of claim 1, comprising the further step of adjusting the tonicity of the solution to a physiologic value under such conditions that the ionic strength is not appreciably altered.
13. The method of claim 12, wherein the tonicity of the solution is adjusted by adding a carbohydrate to the solution.
14. The method of claim 13, wherein the carbohydrate used is maltose.
15. The method of claim 12, wherein the tonicity of the solution is adjusted to a range of about 230 to about 490 mosmol/kg solvent.
16. The method of claim 15, wherein the tonicity of the solution is adjusted to a range of about 274 to about 309 mosmol/kg solvent.
17. The method of claim 12, wherein the tonicity of the solution is adjusted by adding an amino acid to the solution.
18. The method of claim 17, wherein the amino acid used is glycine.
19. The method of claim 1, wherein the trialkylphosphate is tri-n-butyl phosphate and the detergent is selected from polysorbate 80 and sodium cholate.
20. The method of claim 1, wherein the solution has a pH between about 3.5 and about 6.0 during step a).
21. An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001 M, a pH between about 3.5 and about 5.0, an antibody concentration of about 5% wt./wt., and a maltose concentration of about 10% wt./wt.
22. The preparation of claim 21, wherein the pH is about 4.25.
23. An intravenously injectable immune serum globulin preparation produced by the method of claim 1 and substantially free of lipid enveloped viruses, wherein the preparation has an ionic strength less than about 0.001, a pH between about 3.5 and about 5.0, an antibody concentration of about 10% wt./wt., and a glycine concentration of about 0.2 M.
24. The preparation of claim 23, wherein the pH is about 4.25.

\* \* \* \* \*

**EXHIBIT B**



**Blood | Vaccines | Cellular/Gene Therapy | Tissue | Devices**  
**Products | Industry | Healthcare | Reading Room | Meetings | What's New**

## Product Approval Information - Licensing Action

DEPARTMENT OF HEALTH AND HUMAN SERVICES  
Public Health Service  
Food and Drug Administration  
Rockville, MD 20852-1448

April 27, 2005

Our STN: BL 125105/0

Baxter HealthCare Corporation  
Baxter BioScience  
Attention: Ms. Angela Blackshere  
Director, Regulatory Affairs  
One Baxter Way  
Westlake Village, CA 91362

Dear Ms. Blackshere:

We have approved your biologics license application for Immune Globulin Intravenous (Human), 10% Solution effective this date. You are hereby authorized to introduce or deliver for introduction into interstate commerce, Immune Globulin Intravenous (Human), 10% Solution under your existing Department of Health and Human Services U.S. License No. 140. Immune Globulin Intravenous (Human), 10% Solution is indicated for primary immune deficiency.

Under this authorization, you are approved to manufacture Immune Globulin Intravenous (Human), 10% Solution at your facility in Lessines, Belgium. You may label your product with the proprietary name GAMMAGARD LIQUID and will market it in 10 mL, 25 mL, 50 mL, 100 mL, and 200 mL fill sizes.

The dating period for Immune Globulin Intravenous (Human), 10% Solution shall be 36 months from the date of manufacture when stored at 2-8°C, or not more than 9 months storage at 25°C after not more than 15 months storage at 2-8°C. The date of manufacture shall be defined as the date of final sterile filtration of the formulated drug product. Following the final sterile filtration, no reprocessing/reworking is allowed without prior approval from the Agency. Your drug substance may not be held prior to drug product formulation.

Please submit final container samples of the product in final containers together with protocols showing results of all applicable tests. You may not distribute any lots of product until you receive a notification of release from the Director, Center for Biologic Evaluation and Research (CBER).

You must submit information to your biologics license application for our review and written approval under 21 CFR 601.12 for any changes in the manufacturing, testing, packaging or labeling of Immune Globulin Intravenous (Human), 10% Solution, or in the manufacturing facilities.

All applications for new active ingredients, new dosage forms, new indications, new routes of administration, and new dosing regimens are required to contain an assessment of the safety and effectiveness of the product in pediatric patients unless this requirement is waived or deferred. We have reviewed your submission and agree that a deferral of your pediatric studies for Immune Globulin Intravenous (Human), 10% Solution is justified because of limited enrollment of pediatric subjects in your clinical study.

We acknowledge your written commitment as described in your letters of April 21, 2005 and April 22, 2005 as outlined below:

**Postmarketing Studies subject to reporting requirements of 21 CFR 601.70.**

1. Baxter has committed to providing a pediatric plan within sixty (60) days of obtaining licensure.

Deferred pediatric studies required under section 2 of the Pediatric Research Equity Act (PREA) are considered required postmarketing study commitments. The status of these postmarketing studies shall be reported annually according to 21 CFR 601.70. These commitments are listed below.

- a. Deferred pediatric studies under PREA for the treatment of primary immune deficiency in pediatric patients.
- b. Final Report Submission: May 1, 2009.

Submit final study reports to this BLA. For administrative purposes, all submissions related to these pediatric postmarketing study commitments must be clearly designated "Required Pediatric Study Commitments".

We request that you submit clinical protocols to your IND, with a cross-reference letter to this biologics license application (BLA), STN BL 125105/0. Submit nonclinical and chemistry, manufacturing, and controls protocols and all study final reports to your BLA, STN BL 125105/0. Please use the following designators to label prominently all submissions, including supplements, relating to these postmarketing study commitments as appropriate:

- **Postmarketing Study Protocol**
- **Postmarketing Study Final Report**
- **Postmarketing Study Correspondence**
- **Annual Report on Postmarketing Studies**

For each postmarketing study subject to the reporting requirements of 21 CFR 601.70, you must describe the status in an annual report on postmarketing studies for this product. The status report for each study should include:

- information to identify and describe the postmarketing commitment,
- the original schedule for the commitment,
- the status of the commitment (i.e. pending, ongoing, delayed, terminated, or submitted), and
- an explanation of the status including, for clinical studies, the patient accrual rate (i.e. number enrolled to date and the total planned enrollment).

As described in 21 CFR 601.70(e), we may publicly disclose information regarding these postmarketing studies on our Web site (<http://www.fda.gov/cder/pmc/default.htm>). Please refer to the April 2001 Draft Guidance for Industry: Reports on the Status of Postmarketing Studies – Implementation of Section 130 of the Food and Drug Administration Modernization Act of 1997 (see <http://www.fda.gov/cber/gdlns/post040401.htm>) for further information.

In addition, we acknowledge your written commitments of April 12, 2005 and April 18, 2005 that include the following:



**Postmarketing Studies not subject to reporting requirements of 21 CFR 601.70.**


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In addition, pursuant to 21 CFR 600.80(c)(2)(Periodic Adverse Experience Reports), the Agency is requiring that manufacturers report on a monthly basis any infectious disease transmission associated or possibly associated with any licensed biological product that is not reportable under 21 CFR 600.80 (c)(1)(Fifteen-day Alert Reports). The timing of this monthly periodic reporting requirement was selected, among other reasons, to permit the acquisition of patient information, including clinical evaluation, sufficient to help in the timely assessment of a causal connection between the biological product and possible or documented infectious disease transmission. This new reporting requirement was also based on the observation of inconsistent practices by some manufacturers in submitting reports of possible infectious diseases.

Please note that this monthly reporting requirement applies only to infectious disease transmission. Other periodic reports should continue to be submitted on the quarterly or annual basis that is appropriate to each licensed biological product for all other adverse experiences not reportable under 21 CFR 600.80(c)(1). You should submit these monthly reports to The Center for Biologics Evaluation and Research, Division of Epidemiology, HFM-210, 1401 Rockville Pike, Rockville, MD, 20852-1448. Please contact the Division of Epidemiology (301-827-3974) if you have any questions about these periodic adverse event reporting requirements.

You must submit adverse experience reports under the adverse experience reporting requirements for licensed biological products (21 CFR 600.80) and you must submit distribution reports under 21 CFR 600.81. You should submit postmarketing adverse experience reports and distribution reports to the Center for Biologics Evaluation and Research, HFM-210, Food and Drug Administration, 1401 Rockville Pike, Rockville, MD 20852-1448. Prominently identify all adverse experience reports as described in 21 CFR 600.80.

You must submit reports of biological product deviations under 21 CFR 600.14. You promptly should identify and investigate all manufacturing deviations, including those associated with processing, testing, packing, labeling, storage, holding and distribution. If the deviation involves a distributed product, may affect the safety, purity, or potency of the product, and meets the other criteria in the regulation, you must submit a report on Form FDA-3486 to the Director, Office of Compliance and Biologics Quality, Center for Biologics Evaluation and Research, HFM-600, 1401 Rockville Pike, Rockville, MD 20852-1448.

Please submit all final printed labeling at the time of use and include implementation information on FDA Form 356h and FDA Form 2567 as appropriate. Please provide a PDF-format electronic copy as well as original paper copies (ten for circulars and five for other labels). In addition, you may wish to submit two draft copies of the proposed introductory advertising and promotional labeling with an FDA Form 2253 to the Center for Biologics Evaluation and Research, Advertising and Promotional Labeling Branch, HFM-602, 1401 Rockville Pike, Rockville, MD 20852-1448. Two copies of final printed advertising and promotional labeling should be submitted at the time of initial dissemination, accompanied by a FDA Form 2253.

All promotional claims must be consistent with and not contrary to approved labeling. You should not make a comparative promotional claim or claim of superiority over other products unless you have submitted data to support such claims to us and had them approved.

Sincerely yours,

--- signature ---

Basil Golding, M.D.  
Director  
Division of Hematology  
Office of Biologics Research and Review  
Center for Biologics Evaluation and Research

Updated May 9, 2005

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## FDA Approves Baxter's GAMMAGARD Liquid 10% for Patients with Primary Immunodeficiency Disorders

**Formulation, purification and packaging advancements provide benefits to healthcare professionals and patients**

DEERFIELD, Ill., May 2, 2005 – Baxter Healthcare Corporation announced today that the U. S. Food and Drug Administration (FDA) has approved GAMMAGARD® Liquid [Immune Globulin Intravenous (Human)] (IGIV) 10% Solution for the treatment of primary immunodeficiency disorders associated with defects in humoral immunity. Primary immunodeficiency is a group of genetic disorders in which the immune system fails to produce adequate amounts of antibodies, thereby predisposing individuals to increased risk of infection. The company plans to launch the plasma-based therapy in the fourth quarter of this year.

GAMMAGARD Liquid 10% offers improved convenience for healthcare professionals and patients. The ready-to-use, sterile preparation of GAMMAGARD Liquid 10% eliminates the need for reconstitution. In addition, its high concentration, compared to 5% concentrations, allows for a reduction in the length of infusion, reducing the infusion volume and saving time for both patients and healthcare professionals.

The safety of GAMMAGARD Liquid 10% has been demonstrated in a wide spectrum of patients with primary immunodeficiency disorders. Baxter produces the therapy using a three-step viral reduction process, a unique combination used to help ensure viral safety. GAMMAGARD Liquid 10% is free of added sugar, sodium, and preservatives. In addition, the packaging is latex-free.

"The approval of GAMMAGARD Liquid 10% is an exciting milestone in the treatment of primary immunodeficiency disorders, allowing for increased convenience to help patients best manage their health," said Fred Modell, president and co-founder, Jeffrey Modell Foundation.

"As researchers continue to increase their knowledge and understanding of primary immunodeficiency disorders, the approval of this next generation IGIV will truly benefit patients with these disorders," said Marcia Boyle, chairman and chief executive officer, Immune Deficiency Foundation. "The Immune Deficiency Foundation applauds Baxter for its commitment to providing therapies for our community."

The approval was based on a Phase III, multicenter study of 61 patients between the ages of 6 and 72 years who were treated with 300 to 600 mg/kg every 21 to 28 days for 12 months. In this study, no validated acute serious bacterial infections occurred in any of the treated subjects.

"During the clinical trial, we found that GAMMAGARD Liquid 10% was safe and effective in the treatment of patients with primary immunodeficiency disorders," said Joseph Church, M.D., Professor of Clinical Pediatrics, Keck School of Medicine at the University of Southern California, Head of the Division of Clinical Immunology and Allergy at Childrens Hospital Los Angeles and a lead investigator in the clinical trial. "In addition, the production of GAMMAGARD Liquid 10% incorporates a three-step viral reduction process that may improve viral safety."

Baxter intends to launch GAMMAGARD Liquid 10% in five vial sizes (1g, 2.5g, 5g, 10g and 20g). The various vial sizes will allow for tailored dosing and help reduce waste.

Healthcare institutions will also benefit from the therapy's recommended storage conditions. GAMMAGARD Liquid 10% can be stored for up to nine months at room temperature, or for up to 36 months if kept under refrigeration.

"We developed this next generation IGIV therapy in direct response to the needs of patients and healthcare professionals," said Joy Amundson, president of Baxter's BioScience business. "We are proud to add this important innovation to Baxter's 50-year history and commitment to safe and effective plasma therapies."

#### **About GAMMAGARD Liquid 10%**

GAMMAGARD Liquid 10% is a ready-to-use, sterile preparation of highly purified and concentrated immunoglobulin G (IgG) antibodies. GAMMAGARD Liquid 10% is processed from human plasma and contains a broad spectrum of IgG antibodies against bacterial and viral agents. The quality of GAMMAGARD Liquid 10% begins with the donor selection process and continues throughout plasma collection, which only occurs at FDA-approved blood establishments, and plasma preparation. To further support the margin of safety, three validated, independent and effective virus inactivation/removal steps have been integrated into processing and formulation, namely solvent/detergent (S/D) treatment, 35 nanometer filtration, and a low pH incubation at elevated temperature.

Baxter will continue to supply its current GAMMAGARD S/D to those patients who require a low Immunoglobulin A (IgA) therapy.

#### **About Primary Immunodeficiency Disorders**

Primary immunodeficiency disorders encompass more than 100 diseases caused by an immune system that does not function correctly. According to the Immune Deficiency Foundation, approximately 50,000 persons in the United States have one of the primary immunodeficiency disorders. For many people with primary immunodeficiency, the cause is a lack of antibodies. IGIV therapy can help restore IgG levels to near normal, helping the immune system function properly and prevent infections or fight them when they occur.

#### **Important Safety Information**

GAMMAGARD Liquid 10% is contraindicated in patients with known anaphylactic or severe hypersensitivity responses to Immune Globulin (Human). Patients with severe selective IgA deficiency (IgA < 0.05 g/L) may develop anti-IgA antibodies that can result in a severe anaphylactic reaction. Such patients should only receive intravenous immune globulin with utmost caution and in a setting where supportive care is available for treating life-threatening reactions.

**Black Box Warning :** IGIV products have been associated with renal dysfunction, acute renal failure, osmotic nephrosis, and death. While these reports of renal dysfunction and acute renal failure have been associated with the use of many of the licensed IGIV products, those containing sucrose as a stabilizer accounted for a disproportionate share of the total number. GAMMAGARD Liquid 10% does not contain sucrose.

GAMMAGARD Liquid 10% is made from human plasma. Products made from human plasma may carry a risk of transmitting infectious agents, such as viruses, that can cause disease.

The potential risks and benefits of IGIV should be weighed against those of alternative therapies for all patients for whom IGIV administration is being considered.

Please visit [www.baxter.com](http://www.baxter.com) for full prescribing information.

#### **About Baxter**

Baxter Healthcare Corporation is the principal U.S. operating subsidiary of Baxter International Inc. (NYSE: BAX). Baxter International Inc., through its subsidiaries, assists

healthcare professionals and their patients with treatment of complex medical conditions, including cancer, hemophilia, immune disorders, kidney disease and trauma. The company applies its expertise in medical devices, pharmaceuticals and biotechnology to make a meaningful difference in patients' lives. For more information about Baxter, please visit [www.baxter.com](http://www.baxter.com).

*Statements in this press release including but not limited to Baxter's intention to launch GAMMAGARD Liquid 10% in the fourth quarter of this year are forward-looking statements that involve risks and uncertainties. Actual results could differ materially from the above forward-looking statements as a result of certain factors, including the risk and uncertainty related to actions of regulatory bodies and other government authorities, including the FDA and foreign counterparts that could delay, limit or suspend product sales and distribution; product quality and/or patient safety concerns leading to product recalls, withdrawals, launch delays or declining sales; product development risks; technological advances in the medical field; demand for and market acceptance risks for new and existing products and other technologies; reimbursement policies of government agencies and private payers; internal and external factors that could impact commercialization; and other risks detailed in the company's filings with the Securities and Exchange Commission. The company disclaims any current intention to update any forward-looking statements as a result of new information, future events, changed assumptions or otherwise, and all forward-looking statements speak only as of the time when made. Actual results or experience could differ materially from the forward-looking statements.*

#### For Additional Information

**Package Insert**  
GAMMAGARD Liquid  
Package Insert (pdf 144k)

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**EXHIBIT D**



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## News Release

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### Baxter Announces the U.S. Launch of Gammagard Liquid 10% For Patients With Primary Immunodeficiency Disorders

DEERFIELD, Ill., September 26, 2005 – Baxter Healthcare Corporation announced today that GAMMAGARD LIQUID™ [Immune Globulin Intravenous (Human)] (IVIG) 10% Solution is now available to patients in the United States for the treatment of primary immunodeficiencies. Primary immunodeficiency is a group of genetic disorders in which the immune system fails to produce adequate amounts of antibodies, thereby predisposing individuals to increased risk of infection.

"The launch of GAMMAGARD LIQUID is the latest step in Baxter's ongoing efforts toward advancing the science of IVIG," said Joy Amundson, president of Baxter's BioScience business. "We are proud to provide this next generation IVIG therapy to address the needs of healthcare professionals and patients with primary immunodeficiency disorders."

GAMMAGARD LIQUID offers improved convenience for healthcare professionals and patients. The therapy's ready-to-use, sterile preparation eliminates the need for reconstitution. In addition, its high concentration, compared to 5% concentrations, allows for a reduction in the length of infusion, reducing the infusion volume and saving time for both patients and healthcare professionals. It is also the first and only 10% IVIG solution with the following unique combination of features: no added sugars, no added sodium, no added preservatives and latex-free packaging.

In the U.S., GAMMAGARD LIQUID is available in five vial sizes (1g, 2.5g, 5g, 10g and 20g) and can be stored for up to nine months at room temperature within 24 months of manufacture, or for up to 36 months if kept under refrigeration. The therapy is processed from human plasma and contains a broad spectrum of IgG antibodies against bacterial and viral agents. To help ensure viral safety, three validated, independent and effective virus inactivation/removal steps have been integrated into processing and formulation, namely solvent/detergent (S/D) treatment, 35 nanometer filtration, and low pH incubation.

Baxter will continue to supply its current GAMMAGARD S/D only for use with patients who require a low Immunoglobulin A (IgA) therapy.

Outside the United States, Baxter has also submitted applications for marketing authorization to the European Medicines Agency (EMA), Swissmedic and Health Canada's Drug Directorate.

#### About Primary Immunodeficiency Disorders

Primary immunodeficiency disorders encompass more than 100 diseases caused by an immune system that does not function correctly. According to the Immune Deficiency Foundation, approximately 50,000 persons in the United States have one of the primary immunodeficiency disorders. IVIG therapy can help restore IgG levels to near normal, helping the immune



system function properly and prevent infections or fight them when they occur.

### Important Safety Information

GAMMAGARD LIQUID is contraindicated in patients with known anaphylactic or severe hypersensitivity responses to Immune Globulin (Human). Patients with severe selective IgA deficiency (IgA < 0.05 g/L) may develop anti-IgA antibodies that can result in a severe anaphylactic reaction. Such patients should only receive intravenous immune globulin with utmost caution and in a setting where supportive care is available for treating life-threatening reactions.

IVIG products have been associated with renal dysfunction, acute renal failure, osmotic nephrosis, and death. While these reports of renal dysfunction and acute renal failure have been associated with the use of many of the licensed IVIG products, those containing sucrose as a stabilizer accounted for a disproportionate share of the total number. GAMMAGARD LIQUID does not contain sucrose.

GAMMAGARD LIQUID is made from human plasma. Products made from human plasma may carry a risk of transmitting infectious agents, such as viruses, that can cause disease.

The potential risks and benefits of IVIG should be weighed against those of alternative therapies for all patients for whom IVIG administration is being considered.

### About Baxter

Baxter Healthcare Corporation is the principal domestic operating subsidiary of Baxter International Inc. (NYSE: BAX). Baxter International Inc., through its subsidiaries, assists healthcare professionals and their patients with treatment of complex medical conditions, including cancer, hemophilia, immune disorders, kidney disease and trauma. The company applies its expertise in medical devices, pharmaceuticals and biotechnology to make a meaningful difference in patients' lives.

For more information about Baxter, including [GAMMAGARD LIQUID full prescribing information](#), please visit [www.baxter.com](http://www.baxter.com).

[Please See Full Important Safety Information.](#)

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